IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Matthias Giese *et al.*	Art Unit:	1648
Serial No.:	10/675,444	Examiner:	Louise Wang Zhiying Humphrey
Filing Date:	September 30, 2003	Atty. Docket No.	103-001PUS
Title:	Equine Arteritis Virus Vaccine	Confirmation No.	7837

DECLARATION UNDER 37 C.F.R. § 1.132

Dear Dr. Humphrey:

Responsive to the Final Office Action mailed on October 16, 2008, I, Matthias Giese, declare and state that:

1. I am a citizen of Germany residing at Im Schaffner 24, Heidelberg, Germany, D69123.

2. I am presently Head of Department for Vaccine Development at the Frauenhofer Institute for Cell Therapy and Immunology in Leipzig, Germany and also an Assistant Professor in the Faculty of Veterinary Medicine at the University of Leipzig, Germany. A *curriculum vitae* summarizing my educational background and professional experience was previously submitted with a Declaration in this case dated July 18, 2008 and is thus already a matter of record.

3. I am a co-inventor and sole Assignee of the subject-matter disclosed by 10/675,444 entitled "Equine Arteritis Virus Vaccine." I am familiar with the Application and its pending claims, and have reviewed the non-final Office Action of October 16, 2008 (herein "Office Action"). I am also aware of the substance of the telephonic interview between my professional representative and the U.S. PTO that occurred on January 8, 2009.

4. The instant invention relates to a nucleic acid-based prophylactic / therapeutic vaccine against EAV-associated diseases. In particular, the invention describes a vaccine composition and nucleic acid vectors (and related methods) which render an animal, such as a horse, immune against an invasion from an equine arterivirus (EAV) by, for example, inducing a cellular immune response. The vaccine composition / nucleic acid vectors used consisted of EAV open reading frame nucleic acids (ORF) 2, SEQ ID NO:5, SEQ ID NO:9 (ORF 5), and SEQ ID NO:7 (ORF 7) (noted in claims 1 and 15).

5. I understand that in the Office Action, the Examiner rejected the claims, wholly, or in part, as "being prima facie obvious to one of ordinary skill in the art at the time the invention was made" considering the teachings of both Toplasch and Snijder together and also in various combinations with Krieg, Cantion, and Gregoriadis (Office Action, pages 6, 7, 8 and 9, respectively).

6. Based on the argumentation provided in the Office Action (e.g. on pages 4-6), it is my opinion that additional clarification is required by me, as an inventor of the disclosed subject-matter, both to explain the unique technical aspects of our invention and how such aspects are different, and represent improvements over, what was known in the prior art at our invention date.

7. For the reasons described in the following paragraphs, I consider that the claimed invention would not have been obvious to me or to a scientist having ordinary skill in the fields of virology and immunology including knowledge of vaccine development, biochemistry, recombinant technologies, or the like:

8. The EAV genome is particularly infectious and contains at least eight open reading frames (ORF) including ORFs 1a, 1b, 2, 3, 4, 5, 6, and 7. ORFs 2 to 7 are overlapping and are situated at the 3'-end of EAV genome. These EAV ORFs encode various proteins, including structural viral proteins, for example, a phosphorylated nucleocapsid protein ("N", 14 kDa, gene product of ORF 7), an N-glycosylated major membrane protein ("G_L", 30-44 kDa gene product of ORF 5), and an unglycosylated membrane protein ("M", 17 kDa gene product of ORF 6). Moreover, the gene products

derivable from the ORF 2 sequence include an N-glycosylated minor membrane protein ("G_S"), and an envelope protein ("E") (Snijder *et al.*, 1999).

9. Immunization with plasmid DNA, including the EAV compositions and vectors of our invention, which express foreign antigens may provoke both a cellular and humoral immune response, which provides optimal protection against most virally caused infectious diseases. Of note, antigen-specific cytotoxic T-lymphocyte activity is largely responsible for the elimination of (virally) infected cells ("cellular immunity"), while secreted antibodies may also bind to antigens (such as free virus), which thus flags these cells for lysis /destruction ("humoral immunity"). This double-pronged attack affords an organism with a high degree of protection against a viral invasion. Viruses can however, surreptitiously evade attack by the immune system and may establish persistent infections. Such viral persistence can result from several mechanisms, including high genetic variability / instability of a viral genome, interference with cellular machinery, or depletion of immunocompetent cells.

10. To emphasize, the subject-matter of our invention relates to an EAV-derived vaccine composition (and nucleic acid vectors) consisting of the combination of EAV ORFs 2, 5, and 7 having the described sequences, wherein this EAV combination successfully induced a stable and long-lasting immune response. All three of these antigens (ORF 2: small glycoprotein GP_S; ORF 5: large envelope glycoprotein GP_L; and ORF 7: nucleocapsid protein N) significantly stimulated cellular immunity in an antigen specific manner.

11. I refer the Examiner to Tables 19 and 20, where we show a measured specific lysis on an "ORF-by-ORF" basis for all of the horses, which were immunized by intramuscular injection and intradermal application using a gene gun (see [0251]). To estimate the specific lysis, we calculated for each ORF (i.e. ORF 2, ORF 5, and ORF 7), the average value (X) of the specific lysis of all different effector / target cell ratios (3:1, 25:1, 50:1) and subtracted the average value (Y) of the negative controls (measurements taken before immunization). The formula we used is indicated in the Application at [0263] and in the legend for Tables 19 & 20 at [0387] and [0388]. The results depicted in these Tables show an absolute increase of cell lysis at the indicated

times for each ORF compared to the values obtained before immunization. Two different scales were used to express this observed increase of specific lysis, namely in Table 19, a "+" indicates an increased specific lysis of 0-5%, while in Table 20, a "—" indicates an increased specific lysis of 0-5%. For both Tables, each further "+" entry indicates a further 0-5% increase in specific lysis (see [0265] and the legends to the Table 19 & 20).

12. I consider that an important aspect of our invention is the novel configuration of EAV ORF sequences that, as explained above, leads to both a sustained cellular and humoral immune response in horses vaccinated with our compositions. Important considerations for this vaccine design included our observations that the EAV ORF 5 encoded viral membrane protein has been shown to be a powerful immunogen promoting humoral immunity; the ORF 7 encoded viral capsid protein provokes a powerful cytotoxic response; and that the minor ORF 2 membrane protein initiated at least some cytotoxic T-cell response as well (previously unpublished data, but see ANNEX A). We thus included the ORF 2 in our EAV vaccine combination because: it is highly conserved in the *Arterivirus* family and could advantageously serve as a stable "back-up" immunogen to the relatively unstable, but potent ORF 5 membrane protein, which has a high mutation rate—despite the fact that the EAV ORF genome exists at a comparatively minor percentage in the EAV genome (1-2%), and thus it was not expected to generate an immune response worthy of its inclusion in a vaccine.

13. Accordingly, the naturally low EAV ORF 2 antigen concentration was thought by scientists in this field to contribute to the extremely poor antigenic recognition of ORF 2 protein, i.e. limited antibody response, that was observed in one mouse B-cell model (Chimside, of record). This observation may explain why, until now, the use of ORF 2 with other ORFs in a vaccine composition has not been pursued. This, in part, explains our statement in [0057] in the Application where we note that it was "surprising" and "contrary to the opinion in the art" that our vaccine composition comprising the three above-mentioned ORFs provided improved, and longer lasting, sustainable results than studies using the entire EAV cDNA sequence (e.g. Chimside). However, the data presented in Table 17 clearly show a sustained immune response following a

vaccination with our EAV vaccine compositions (humoral response), while Tables 19-20 show an elevated and sustained (cellular) response at the indicated points in time.

14. Another surprising aspect of our invention (as mentioned in the Application at [0053]) was that ORF 7 could, in fact, also be recognized by cytotoxic T-cells, since this ORF encodes for a capsid protein that is situated internally within the virus. This unexpected recognition by, and thus mobilization of, the T-cell response to the ORF 7 antigen may be due to imperfect translation of the mRNA to protein during the viral replication process, which thereby provokes this immune response due to mistakes in the capsid protein assembly.

15. All of the above-described aspects of our inventions, in particular, the underlying scientific principles, provide subject-matter that did not exist in the prior art before our relevant date. Regarding the Snijder publication, I note that Snijder provides us with a characterization of the ORF 2a protein using a mouse model; there is no concrete functional data regarding ORF 2a, in particular no cytotoxic effects of the ORF 2a antigen are demonstrated. Rather, Snijder explains that ORF 2a is a structural component of EAV that is indispensable for generating infectious particles (on page 6336, bottom of column 1). Snijder also notes that the ORF 2a protein may undergo certain post-translational modifications that can alter its function due to changes in its 3D structure (page 6344, middle of column 1). Such changes would most certainly have consequences if present in a vaccine (i.e. possibly jeopardizing the expected vaccine protection), thus further investigation would be required by the skilled person in order to understand what those changes may be and how this might affect the ability of ORF 2a to mobilize an immune response. Finally, Snijder performed a simultaneous expression of EAV ORFs 2a, 5 "and/or" 6 in a vaccinia virus-based expression system, and failed to produce any subviral particles (data not shown) (page 6344, column 2). The failure of this viral vector system, which is quite different from the expression plasmids used in the present invention, could theoretically be due to defective vector expression, which depends on an active virus and is known to have limitations in accepting foreign antigens to thereby generate an immune response.

16. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

DATED: April 2, 2009

Prof. Dr. Matthias Giese